APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1978, p. 290-296
0099-2240/78/0035-0290$02.00/0

Vol. 35, No. 2
Printed in U.S.A.

Airborne Enteric Bacteria and Viruses from Spray Irrigation with Wastewater

B. TELTSCH AND E. KATZENELSON*

Environmental Health Laboratory, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received for publication 19 August 1977

The relationship between bacterial concentrations in wastewater used for spray irrigation and in the air was examined. Aerosolized coliforms were detected when their concentration was 10^3/ml or more in the wastewater. Relative humidity and solar irradiation appeared to affect viable bacteria in the air; a positive correlation was found between relative humidity and the number of aerosolized bacteria. The correlation between solar irradiation and bacterial level, on the other hand, was negative. During night irrigation, up to 10 times more aerosolized bacteria were detected than with day irrigation. Wind velocity did not play an important role in the survival of aerosolized bacteria. Echovirus 7 was isolated in 4 out of 12 air samples collected 40 m downwind from the sprinkler.

Agricultural spray irrigation with sewage effluents, as a way to increase the water potential and as an important alternative to advanced wastewater treatment, is widely practiced throughout the world.

One of the disadvantages of spray irrigation is the aerosolization of pathogens that may be present in domestic sewage (2, 11) even after secondary treatment and chlorination (12). Essentially, very few quantitative data are available to evaluate the possible public health risks from these pathogen-containing aerosols (3, 6, 14, 15). Epidemiological evidence indicates that potential health risks may be involved in the use of wastewater for spray irrigation, since the incidence of enteric communicable diseases was found to be two to four times higher in settlements in Israel irrigating with wastewater than in communities not practicing this form of irrigation (7).

Scarcely any work has been reported on the dissemination of bacteria and viruses from spray irrigation with wastewater effluents. Schultze (10) studied the fallout of small droplets from the watering of crops with liquid raw sewage from an overhead sprinkling irrigation system. He detected *Escherichia coli* by using open petri plates positioned at varying distances downwind. Merz (9) considered viable bacterial travel to be limited to the distance reached by viable mist emanating from the sprinkler. We (8) have found coliform bacteria in the air at a distance of 350 m downwind from a spray irrigation line. In one instance, a *Salmonella* sp. was isolated 60 m from the source of irrigation. Sorber et al. (13), in their study of bacterial aerosols at a wastewater irrigation site, concluded that greater bacterial aerosol concentrations occurred under conditions of relative atmospheric stability and darkness. About 50% of the particles that bore viable bacteria were of human respirable size (range 1.0 to 5.0 μm); chlorination reduced bacterial aerosol levels by close to three orders of magnitude.

In the present study, controlled experiments utilizing marker bacteria were carried out to evaluate the quantitative relationship between enteric bacteria in the effluent used for irrigation and aerosolized bacteria detectable in the air, and to evaluate the effects of some meteorological factors such as relative humidity, temperature, wind velocity, and solar irradiation on bacterial dispersion in the air. In addition, attempts were made to examine the air in the vicinity of sewage-irrigated farmland for the presence of human enteric viruses.

MATERIALS AND METHODS

Sampling locations. The air in the vicinity of an effluent-irrigated field located in a small valley near an agricultural school at Ein Kerem was sampled (Fig. 1). The source of the effluent is a large university hospital, the sewage of which undergoes treatment which includes primary sedimentation, trickling filter, sand filtration, and chlorination. After disinfection, the effluent is kept in an operational storage reservoir for a number of days. The quality of the effluent from the reservoir was found to be typical for the type of treatment (biological oxygen demand, 30 mg/liter). The quantity of coliforms in this effluent was 10^3 to 10^5/ml. The irrigation line consisted of one sprinkler (Na'an type no. 233/92, Na'an Metal Works, Israel) with an orifice diameter of 0.5 cm and an output of 1.7 m^3/h. Water pressure was 4.0 atmospheres, and spray height reached about 2.5 m above ground level.

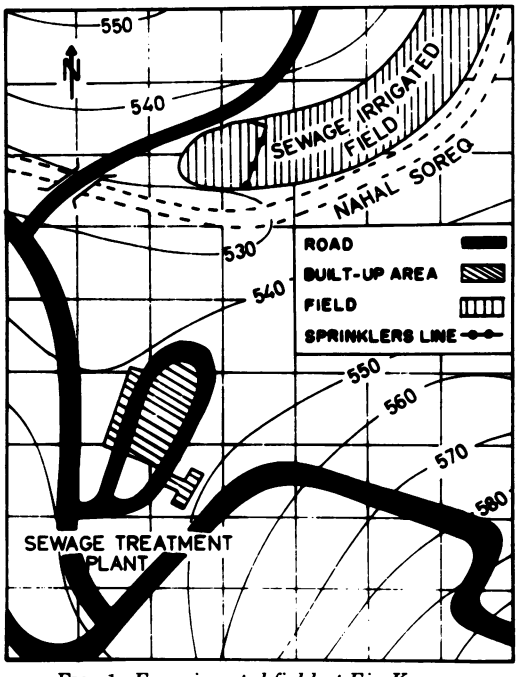

FIG. 1. *Experimental field at Ein Kerem.*

Another sampling site was located near Kibbutz (collective settlement) Tsorah. The site and its effluent are described elsewhere (8).

Marker bacteria. A mutant *E. coli* resistant to the antibiotic nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) was added to the wastewater as a marker bacterium. The mutant was isolated from domestic wastewater by A. Sadovski, Faculty of Agriculture, Rehovot, The Hebrew University.

The marker *E. coli* was grown in 50 liters of nutrient broth (Difco Laboratories, Detroit, Mich.) containing 0.2 mg of nalidixic acid per ml; aeration was provided by bubbling with sterile air during incubation for 72 h at room temperature. The 50 liters of cells and broth was added to a tank that contained 500 liters of effluent. This cell-broth-effluent mixture was introduced constantly into an irrigation line by a water-operated fertilizer pump (T.M.B. Fertilizer Pumps Ltd., Kiryat Bialik, Israel). The rate of introduction was 50 liters/h, providing a concentration of about 10^5 bacteria per ml in the sprayed sewage.

Air sampling. Andersen stacked sieve samplers (Andersen 2000 Inc., Atlanta, Ga.), viable type, were loaded with plastic petri plates containing media of sufficient depth to allow about 2.5-mm clearance between agar and sieve. Sampling time was 10 to 30 min, and the air flow through the samplers was 28.3 liters/min.

A large-volume aerojet-general liquid scrubber (4) was used for virus determination in the air. Sampling time was 15 to 20 min. Collecting fluids were either distilled water or minimum essential medium without antibiotics. The air flow was 600 liters/min, and collection fluid output was 3 ml/min. The samples were kept on ice during transport to the laboratory, where they were stored at −80°C until inoculated into cell cultures.

Microbiological analysis. The mutant bacteria were determined by direct sampling or by the pour plate technique on violet red bile agar (Difco) with 0.2 mg of nalidixic acid per ml. The plates were incubated at 37°C for 24 h.

Total coliform levels in the air and in wastewater were estimated in Endo broth (Difco) as described previously (8).

For virus determination from the air, 20-ml samples were inoculated into Buffalo Green Monkey (BGM) cells (1) grown in 250-ml tissue culture flasks (Falcon Plastics, Oxnard, Calif.). Before inoculation, the following were added to the collection fluids: to distilled water, 2 ml of 10× concentrated sterile minimum essential medium, 0.5 ml of fetal bovine serum, and 0.2 ml of antibiotic solution consisting of, per ml, penicillin 200,000 U, streptomycin 200 mg, kanamycin 200 mg, and neomycin 25 mg; to minimum essential medium, 0.5 ml of fetal bovine serum and 0.2 ml of the antibiotic solution.

Inoculated tissue cultures were incubated at 37°C for 7 days. When cytopathic effect was observed in an inoculated culture, 0.5 ml of the medium was transferred to a fresh culture. A sample was considered positive for viruses only in instances where cytopathic effect was observed in three consecutive transfers. Typing of the isolates was done by neutralization with specific antisera.

Meteorological monitoring. A mechanical wind recorder, Woelfle type (Wilh. Lambrecht, Gottingen, W. Germany), measured wind velocity and direction at the experimental site. Temperature, relative humidity, and solar irradiation figures were obtained from the Laboratory of Climatology and Meteorology, Jerusalem, and from the equipment stationed at the site.

RESULTS

Aerosolization experiments with marker *E. coli*. Results of a typical experiment are summarized in Table 1. Air samples were taken continuously at a distance of 20 m downwind from the sprinklers, with a sampling time of 15

TABLE 1. *Relationship between the concentration of marker E. coli in effluent and in the air during irrigation with contaminated water*

Time of sample[a]	Sample no.	Total coliforms		Marker *E. coli*	
		Bacteria per ml of effluent	Bacteria per m^3 of air	Bacteria per ml of effluent	Bacteria per m^3 of air
Before	1	2.6×10^4	7.0	0	0
During	2			3.4×10^4	7.0
	3			2.5×10^5	3.0
	4			2.4×10^5	12.0
	5	5.6×10^5		3.0×10^5	17.0
	6			5.1×10^5	14.0
	7	3.9×10^5	26.0	3.5×10^5	
After	8			5.7×10^3	0
	9			3.4×10^3	0

[a] Relative to introduction of marker *E. coli* into wastewater.

min. The first air sample was collected before introduction of the marker bacterium, the last two samples after introduction of the marker had been stopped. Mean wind velocity during the experiment was 3.4 m/s, and the temperature was 19°C. The results clearly indicate that the marker bacterium could be detected in the air only after it had been added to the effluent. As soon as the injection of the marker ceased, its concentration in the effluent decreased to 10^3/ml and, at the same time, was undetectable in the air samples.

Effects of meteorological factors. To elucidate the connection between weather conditions and bacterial concentrations, the following experiments were carried out at the Ein Kerem site. Three Andersen samplers, placed 20 m downwind from the sprinkler (Fig. 2), sampled the droplet cloud. The angle between the samplers was 30°. This angle was dictated by the fluctuations in wind direction and the topography of the experimental field. Runs lasted 10 h, with the air being pumped through the three samplers simultaneously every hour. Sampling time was 30 min. Since wind direction fluctuates slightly, only air samples having the highest bacterial counts at each specific sampling time were considered representative for the aerosol levels at the cloud center. Runs in which mean wind direction changed drastically were discarded. At the start and at termination of each sampling period, an effluent sample was taken to determine the average total coliform concentration for the specific run. Average results from four experiments are depicted in Fig. 3. The minimum of the bacterial concentration curve appeared at 12:15 p.m., as did the minimum of the relative humidity curve. The maximum of the irradiation curve appeared at 11:15 a.m. The maximum of the temperature curve was delayed 1 h and that of the wind velocity 3 h with respect to the minimum of the bacterial concentration curve.

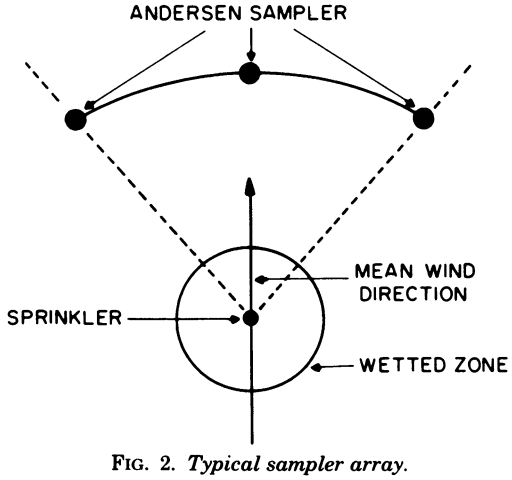

FIG. 2. *Typical sampler array.*

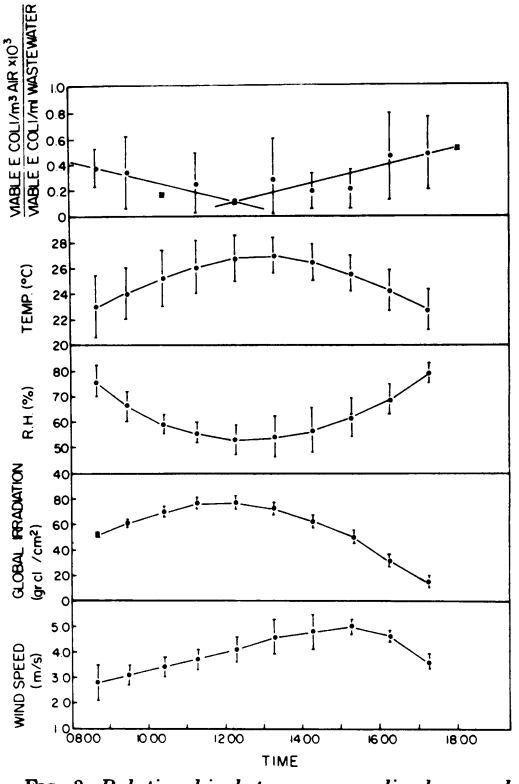

FIG. 3. *Relationship between normalized aerosol concentration, temperature, relative humidity, solar irradiation, and wind velocity during daytime (means and standard deviation of four experiments).*

In a series of identical experiments conducted at night (Table 2), average bacterial concentrations were 10 times higher than during daytime. It should be noted that at night bacterial concentrations in the effluent were similar to or even lower than those during the day.

Sampling for airborne viruses in the vicinity of fields spray-irrigated with sewage. In 4 experiments out of 12, viruses were found in the air at a distance of 40 m downwind from the sprinklers. These positive samples contained echovirus 7.

DISCUSSION

In discussing microbial aerosol generation as a result of spray irrigation with wastewater, it is convenient to group the factors known to affect survival of microorganisms into three major categories: (i) the microorganisms; (ii) aerosol manipulation; and (iii) environment.

In this study, using marker bacteria, bacterial levels in the air were directly related to the bacterial levels in the wastewater sprayed into the air. As the bacterial concentration in the wastewater increased, the probability for discovery of bacteria in the air increased likewise. Reducing the bacterial concentration in the effluent prior to spraying will therefore reduce its level in the surrounding air. Sorber et al. (13) suggested chlorination as a potential and efficient means for controlling the microbial aerosol problem.

Airborne bacteria were found (during daytime) when their concentration in wastewater was 10^3/ml and above. It is reasonable that in air samples larger than those collected in this study, airborne bacteria may be detected even when their concentration in the effluent and in the air is less.

Meteorological conditions directly affected the survival of aerosolized bacteria. Our results suggested a high correlation between the aerosol densities normalized with respect to source strength on the one hand, and relative humidity or solar irradiation on the other. Correlation with wind velocity seemed low, however. To test this hypothesis, regression and correlation between the bacterial concentrations in the air and the meteorological factors were analyzed (Fig. 4–7). The coefficient of correlation with relative

TABLE 2. *Bacterial aerosols during day and night runs*

Expt no.	Wind velocity (m/s)	Solar irradiation (gram calories/cm²)	Relative humidity (%)	Temperature (°C)	*E. coli* concn	
					Effluent (bacteria per ml × 10^5)	Air (bacteria per m^3)
Day						
1	2.9	58.0	60.0	22.7	4.9	70
2	2.8	65.0	56.0	23.4	4.0	94
3	3.2	76.0	53.0	24.7	3.5	91
4	3.4	70.0	50.0	25.5	4.8	21
5	3.6	65.0	47.0	26.0	4.0	44
6	3.9	56.0	48.0	25.0	5.0	40
Mean	3.3	65.0	52.0	24.6	4.3	60
Night						
1	1.5		85.0	21.3	2.1	370
2	0.8		86.0	21.1	1.9	1,130
3	0.8		75.0	21.2	1.9	1,040
4	0.6		63.0	21.0	1.3	700
5	0.6		61.0	20.9	1.3	590
6	0.9		59.0	20.8	1.5	420
Mean	0.9		71.5	21.0	1.6	710

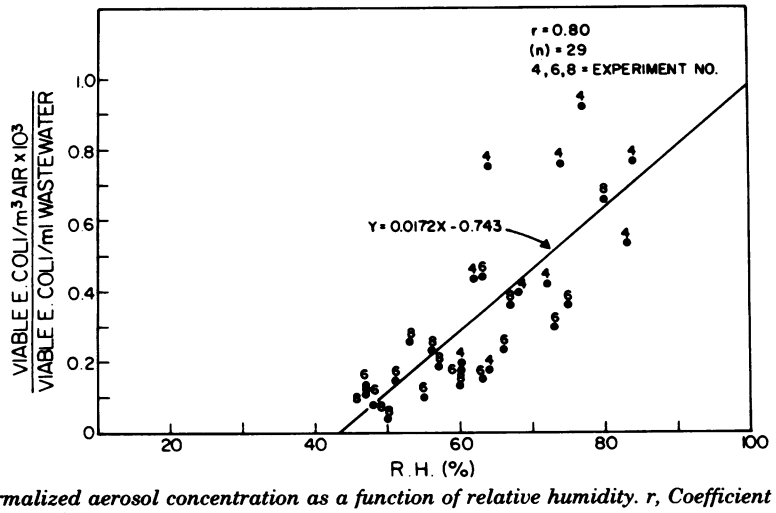

FIG. 4. *Normalized aerosol concentration as a function of relative humidity. r, Coefficient of correlation; (n), number of samples.*

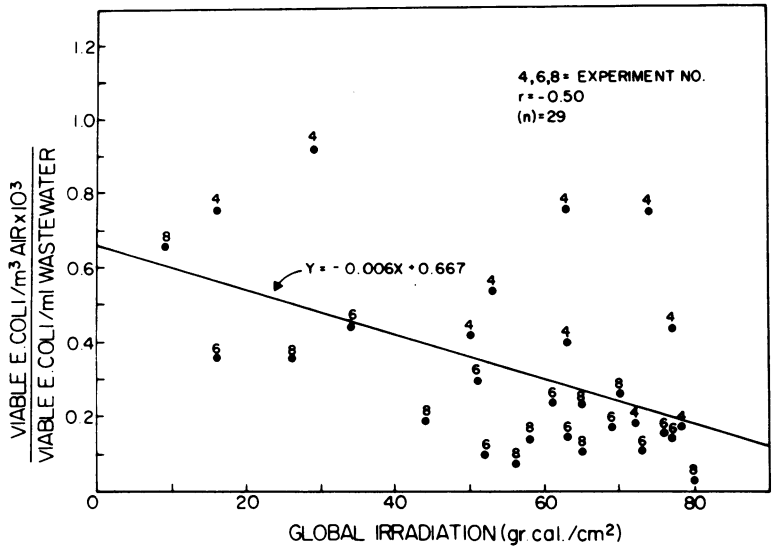

FIG. 5. *Normalized aerosol concentration as a function of solar irradiation. r, Coefficient of correlation; (n), number of samples.*

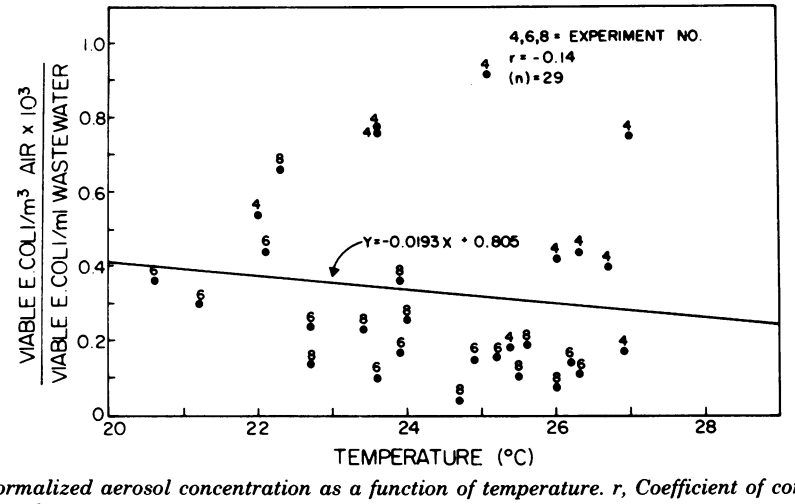

FIG. 6. *Normalized aerosol concentration as a function of temperature. r, Coefficient of correlation; (n), number of samples.*

humidity was high ($r = 0.80$), and it was lower with solar irradiation ($r = -0.50$). Both are significant at the 1% level. There was no correlation with temperature ($r = -0.14$) or with wind velocity ($r = -0.11$).

Since the maximum of the wind velocity lagged about 3 h behind the maximum of the relative humidity (and of solar irradiation), the coefficient of correlation between these two parameters was low ($r = -0.31$), and it was possible to distinguish between their relative effects. From the linear regression between the bacterial densities normalized with respect to source strength and the meteorological factors (Fig. 4–7), it seems that there is a connection between relative humidity levels and aerosol densities, and between solar irradiation and aerosol densities. Relative humidity explains 65% of the variance of the aerosol densities, and the remaining 35% is a deviation from the line.

These analyses show that wind velocity is probably not the major factor in the survival of aerosolized bacteria. Relative humidity and solar irradiation, on the other hand, substantially affect bacterial aerosol levels. At low relative humidity and high solar irradiation, the airborne bacteria are more rapidly inactivated. These results are similar to those obtained with labora-

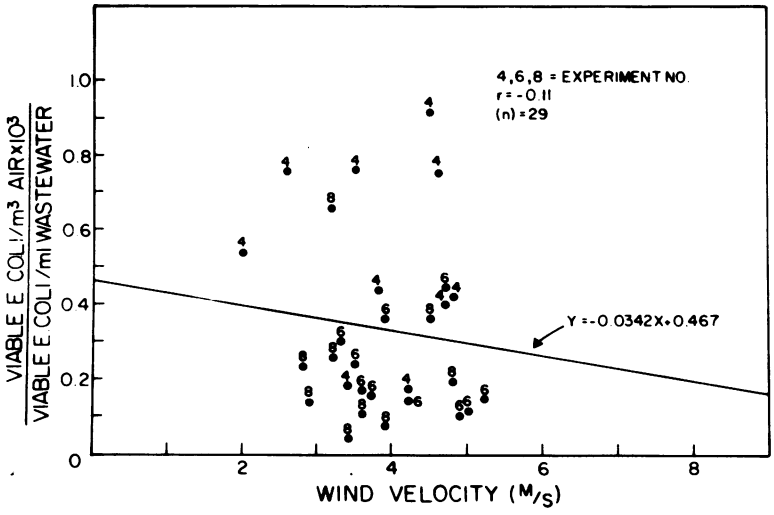

FIG. 7. *Normalized aerosol concentration as a function of wind velocity. r, Coefficient of correlation; (n), number of samples.*

tory experiments in which *E. coli* was aerosolized into air humidified in the range of 45 to 90% relative humidity. The survival rate increased with increasing humidity (5).

During runs performed at night, bacterial aerosol levels were 10 times higher than during daytime. The night runs were characterized by greater atmospheric stability, higher relative humidity, lack of solar irradiation, and lower temperatures. It should be mentioned that irrigation during the night is very common.

The mass median particle diameter was bigger than 7 μm. However, about 50% of the bacteria sampled both during day and night runs were associated with particles smaller than 7 μm. It is these smaller particles that are considered to constitute a health hazard, because they penetrate the lower respiratory tract. We believe the larger particles also to be of health significance, since, after having been caught in the upper respiratory tract, they may be subsequently swallowed. Where enteric bacteria and viruses from sewage are concerned, these larger particles may thus be considered to be an even greater health risk.

In 4 of the 12 experiments, viruses were isolated from the air; in each case they proved to be echovirus 7. The experiments in question were carried out over a period of 2 weeks. The fact that only echovirus 7 was isolated may be due either to its presence in the sewage at that particular time in large numbers, or to its resistance to environmental conditions. Whatever the reason, the presence of human enteric viruses in the air should be cause for awareness.

This study does not prove that enteric bacterial and viral diseases are transmitted through the air as a result of spray irrigation with sewage. It strongly indicates, however, that such a possibility exists.

ACKNOWLEDGMENTS

We thank A. Sadovski, Faculty of Agriculture, Rehovot, Hebrew University, for the marker bacteria; M. Nishmi, Hebrew University-Hadassah Medical School, Jerusalem, and the Virus Laboratory of the Ministry of Health, Tel Aviv, for the virus typing; and D. Ashbel, Laboratory of Climatology and Meteorology, Jerusalem, for the meteorological data.

This study was carried out under a research grant from the Office of the Water Commissioner, Ministry of Agriculture and Ministry of the Interior, Israel, and under grant no. RF-75067 from the Rockefeller Foundation.

LITERATURE CITED

1. **Barron, A. L., G. Olshevsky, and M. M. Cohen.** 1970. Characteristics of BGM line of cells from African green monkey kidney. Brief report. Arch. Gesamte Virusforsch. **32**:389-392.
2. **Chambers, C. N.** 1971. Chlorination for control of bacteria and viruses in treatment of plant effluents. J. Water Pollut. Control Fed. **43**:228-241.
3. **Clark, C. S., E. J. Cleary, G. M. Schiff, C. C. Linnemann, Jr., J. P. Phair, and T. M. Briggs.** 1976. Disease risks of occupational exposure to sewage. J. Environ. Eng. Div. Am. Soc. Div. Eng. **102**:375-388.
4. **Decker, H. M., L. M. Buchanan, D. E. Frisque, M. E. Filler, and C. M. Dahlgren.** 1969. Advances in large volume air sampling. Contamination Control **8**:13-17.
5. **Dimmick, R. L., and A. B. Akers.** 1969. An introduction to experimental aerobiology, p. 280. Wiley-Interscience, New York.
6. **Hickey, J. L. S., and P. C. Reist.** 1975. Health significance of airborne microorganisms from wastewater treatment process. Part II. J. Water Pollut. Control Fed. **47**:2758-2773.
7. **Katzenelson, E., I. Buium, and H. I. Shuval.** 1976. Risk of communicable disease infection associated with wastewater irrigation in agricultural settlements. Science **194**:944-946.

8. **Katzenelson, E., and B. Teltsch.** 1976. Dispersion of enteric bacteria by spray irrigation. J. Water Pollut. Control Fed. **48**:710–716.
9. **Merz, R. C.** 1957. Third report on the study of wastewater reclamation and utilization. Publication 18. California State Water Pollution Control Board, Sacramento.
10. **Schultze, K.** 1943. Untersuchungen zur landwirtschaftlichen Verwertung städtischen Abwassers. Nachweis, Flugweite und physikalische Zustand versprühter Regnertropfen. Arch. Hyg. Bakteriol. **130**:244–263.
11. **Shuval, H. I.** 1970. Detection and control of enteroviruses in the water environment, p. 47–71. *In* H. I. Shuval (ed.), Development in water quality research. Ann Arbor Humphrey Science Publ., London.
12. **Shuval, H. I., J. Cohen, and R. Kolodney.** 1973. Regrowth of coliform and fecal coliforms in chlorinated wastewater effluent. Water Res. **7**:537–546.
13. **Sorber, C. A., H. T. Bausum, S. A. Schaub, and M. J. Small.** 1976. A study of bacterial aerosols at a wastewater irrigation site. J. Water Pollut. Control Fed. **48**:2367–2379.
14. **Sorber, C. A., S. A. Schaub, and H. T. Bausum.** 1974. An assessment of potential virus hazard associated with spray irrigation of domestic wastewaters, p. 241–252. *In* J. F. Malina and B. P. Sagik (ed.), Virus survival in water and wastewater systems. University of Texas, Austin.
15. **Stanford, G. B., and R. Tuburan.** 1974. Morbidity risk factors from spray irrigation with treated wastewater, p. 56–64. *In* Wastewater use in the production of food and fiber proceedings. EPA-660/2-74-041, U.S. Environmental Protection Agency, Washington, D.C.